

## Lack of imposex in the muricid *Morula granulata* from Rowley Shoals, northwestern Australia

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### Abstract

Dissection of 197 specimens of the thaid *Morula granulata* from Rowley Shoals, on the outer continental shelf 300 km west of Broome, northwestern Australia, revealed that none were affected by imposex. While imposex is known to be caused by tributyltin (TBT), there are also other possible causes such as copper, paint matrix and environmental factors. In addition to the relatively low TBT levels in their tissue, the other environmental factors which can cause imposex may also be missing from Bedwell Island.

Key words: imposex, muricid, *Morula granulata*, Australia

### Introduction

Beginning in the late 1960s tributyltin came into widespread use as an antifoulant added to boat paints. The chemical is extremely potent, and concentrations as low as 1 ng TBT l<sup>-1</sup> of seawater have been demonstrated to affect a wide variety of animal phyla. Goldberg (1986) described TBT as "the most toxic substance ever deliberately introduced into natural waters". The best known effect of TBT is the masculinisation of females of dioecious marine gastropods. Blaber (1970) first reported imposex, the phenomenon in which TBT causes the females to begin to develop a penis and/or a vas deferens, in the thaid *Nucella lapillus* (Linnaeus, 1758), in the United Kingdom. In extreme circumstances, the female genital pore becomes occluded and the animal is unable to spawn. This causes considerable population consequences, including the localised extinction of populations (Bryan *et al.*, 1987; Gibbs *et al.*, 1991; Evans *et al.*, 1994). Worldwide, imposex has been reported in over 120 species of marine gastropods (Oehlmann *et al.*, 1996). Many of these species are intertidal muricids. In Western Australia, imposex has been reported in six species of *Conus* (Kohn & Almasi, 1993; Kohn *et al.*, 1999), *Thais orbita* (Gmelin, 1791) (Field, 1993) and *Morula granulata* (Duclos, 1832) (Reitsema & Spickett, 1999; Wells, unpublished).

Rowley Shoals is a series of three isolated oceanic atolls located on the outer continental shelf 300 km west of Broome, northwestern Australia (Figure 1). The atolls are well away from international shipping, and are visited only by a small number of charter vessels and private yachts (Environment Australia, 1999). Passages into the shoals are small and vessels able to navigate the passages are smaller than 25 m in length; use of TBT in boats of this size was banned in Western Australia in 1991. There should be no TBT present at Rowley Shoals, and accordingly no imposex in marine snails caused by TBT.

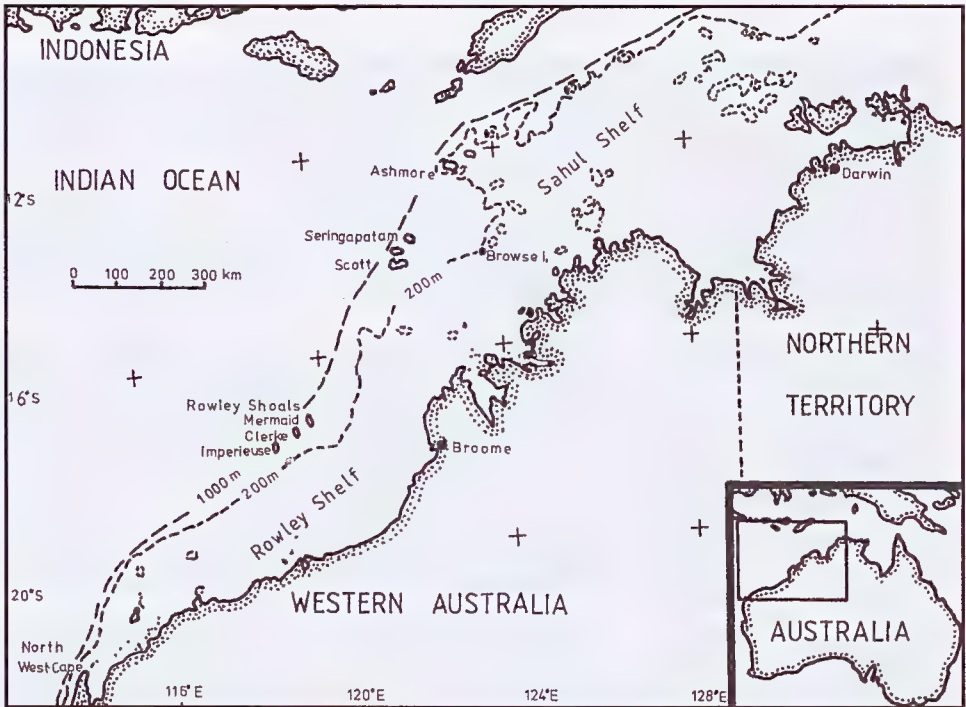


Figure 1.

Map of northwestern Australia showing the location of Rowley Shoals.

### Materials and Methods

To verify this hypothesis, 197 *Morula granulata* were collected from intertidal beachrock at Bedwell Island, Clerke Reef, Rowley Shoals (17°20'S; 119°20'E) on 13 September 1999. Individuals were collected as they were encountered; there was no size selection of the specimens. Animals were frozen until they could be dissected at the Western Australian Museum. Specimens were sorted into adults, with a thickened outer lip and teeth inside the lip, and juveniles. Shell length was measured with Vernier callipers then the shells were cracked off using a bench vice. Sex was determined using the presence or absence of a penis. As development of the penis began to be obvious only at a shell length of 17 mm, only animals of 17 mm or more were sexed. Determination of the presence of imposex was based on the anatomical criteria of Oehlmann *et al.* (1996). A sample of tissue was analysed for TBT by the CSIRO Centre for Advanced Analytical Chemistry using atomic absorption spectrometry.

### Results

*Morula granulata* at Bedwell Island ranged in size from 9 to 25 mm with a mean length of  $18.9 \pm 3.2$  mm (S.D.) (Figure 2). The 67 males averaged  $19.6 \pm 1.8$  mm and the 100 females averaged  $20.2 \pm 2.0$  mm. The difference between mean length of males and females was not statistically significant (*t* test,  $p > 0.05$ ), but females were significantly more abundant than males ( $\chi^2$  test,  $p < 0.05$ ). None of the animals was affected by imposex.

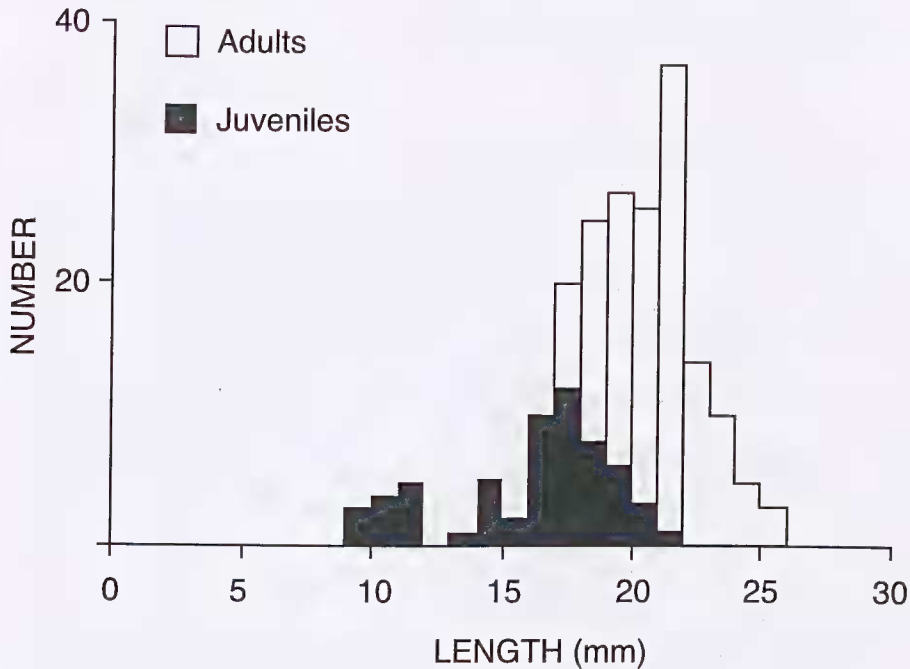


Figure 2.

Size-frequency histogram of *Morula granulata* collected at Rowley Shoals.

The TBT tissue sample was analysed twice by CSIRO. The measured concentration of TBT in the tissues was 30 and 31 ng Sn g<sup>-1</sup>.

### Discussion

*Morula granulata* is known to be affected by imposex in the ports of Dampier and Port Hedland, Western Australia (Reitsemá & Spickett, 1999; Wells, unpublished). The two ports are the largest in Australia in terms of tonnage, and both are heavily utilised by vessels >25 m on which the use of tributyltin is still permitted. Reitsemá & Spickett (1999) recorded from 0 to 57% of animals at Dampier were affected by imposex, and they found a significant correlation with distance to vessel activity. The highest levels of imposex were found in snails with the highest levels of tributyltin (33 ng Sn g<sup>-1</sup>) in their tissues. The level of TBT in *M. granulata* tissues in Dampier is similar to that recorded at Rowley Shoals, but no imposex was found at Rowley Shoals. Wells (unpublished) found 96% and 100% of *M. granulata* at two sites adjacent to wharves in Port Hedland had moderate levels of imposex. In contrast, only 83% of the animals on the Port Hedland foreshore were affected. In contrast to the Western Australian results, Liu *et al.* (1997) reported no imposex in *M. granulata* in Taiwan even though combined tissue concentrations of tributyltin and triphenyltin were as high as 243 ng Sn g<sup>-1</sup>. The mixed results obtained for *M. granulata* in different areas raises the possibility that different populations react in different ways to TBT.

In discussing the population level effects of high TBT concentrations, Evans *et al.* (1994) recorded three major effects:

- reduced recruitment leading to smaller proportions of juveniles to adults;
- reduced abundance; and
- alteration of sex ratios toward male bias.

Juveniles <9 mm were absent and there were few individuals <15 mm of *M. granulata* at Rowley Shoals. This might be taken as evidence that the population is not reproducing. However, the size frequency curve is consistent with data presented by Taylor (1975) for *M. granulata* at several sites at Aldabra Atoll in the Indian Ocean. No data on abundance were collected at Rowley Shoals, and there appear to be no published data on the abundance of *M. granulata* in other localities. However, the fact that 197 individuals could be collected in a single hour at Bedwell Island is evidence that the population density has not been adversely affected by TBT. While the sex ratio of *M. granulata* at Rowley Shoals differs significantly from 50:50, it is biased towards females rather than males. In addition to the lack of imposex, there are thus no characteristics in the population of the type which Evans *et al.* (1994) found to be associated with high levels of TBT.

While TBT has been demonstrated to cause imposex in neogastropods, it is not the only cause. For example, Nias *et al.* (1993) used laboratory experiments to demonstrate that copper, paint matrix and environmental factors may also cause the deformity. The sample of *M. granulata* from the Rowley Shoals is from the only area in the region where the animals are known to occur. In addition to the relatively low TBT levels in their tissues, the other environmental factors which can cause imposex may also be missing from Bedwell Island.

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